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EFFECT OF EDTA ON Pb(II) UPTAKE AND TRANSLOCATION BY TUMBLEWEED (*SALSOLA KALI*): AGAR AND HYDROPONICS STUDIES

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INTRODUCTION

Lead (Pb) has been used since pre-Christian times for its malleability and resistance to corrosion. However, environmental accumulation of this metal represents a worldwide health hazard. Soils are a major sink for lead, which might be absorbed and bioaccumulated by plants and animals eventually becoming available for human consumption. According to the literature, there is a positive correlation between lead in soil and blood lead concentration[1]. Naturally occurring lead in soils is usually found at less than 50 mg kg^{-1}. Nevertheless, contaminated surface soils may contain more than 11,000 mg kg^{-1} [2,3]. Current techniques for removing contaminants from soils are generally expensive and labor intensive. In addition, these techniques are frequently appropriate for small areas and may affect biological activity, soil structure and fertility as well[4]. On the other hand phytoremediation, the use of plants to remove or stabilize contaminants, is an inexpensive technique that may be applied to large areas and to prevent soil erosion.

Plants to be used for phytoremediation purposes should be able to uptake considerable amounts of contaminants, what is known as hyperaccumulation. According to Baker and Brooks[5], a Pb hyperaccumulator plant species should be able to bioconcentrate 1000 mg kg^{-1} Pb in their tissues. However, the low solubility of most lead compounds results

in rare Pb hyperaccumulation. The use of chemical agents to increase metal desorption and solubility from soil has been a helpful tool to enhance metal uptake by plants[6]. According to this, the objective of the present research was to determine the ability of tumbleweed, a desert plant species, to absorb Pb from a contaminated media. In addition, the role of Ethylenediaminetetracetic acid (EDTA) in Pb absorption and translocation was studied using agar and a hydroponic system.

MATERIALS AND METHODS

Agar experiments

For agar experiments, a modified Hoagland's nutrient solution was prepared according to the method used by Peralta *et al.*[7]. A low phosphate concentration was used to avoid Pb(II) precipitation. After sterilization, the media was spiked with $Pb(NO_3)_2$ to obtain different Pb(II) concentrations (5, 20, 50, 75 and 125 mg l^{-1}). EDTA was added in equimolar amounts and chelating agent-free as well as metal-free media were used as control. Agar was added at 0.5% (w/v) and the media was poured into sterilized Mason jars and allowed to solidify. Each treatment was replicated three times for statistical purposes. Tumbleweed seeds collected from areas around El Paso, TX, were treated with an antibiotic-antimycotic solution (Sigma A9909), rinsed with sterilized D.I. water and planted in the media. The jars were placed in a growth chamber with a controlled temperature of 25ºC and a 12-h photoperiod at a photon irradiance of 39.5 µmol $m^{-2} \cdot s^{-1}$. After 15 days of growth, the plants were removed from the growing media and the plant growth as well as the Pb accumulation in roots, stems and leaves was determined.

Hydroponics experiments

For these experiments, tumbleweed seeds were germinated on sterilized paper towels according to the procedure published by Carrillo *et al*.[8]. Seeds were allowed to germinate for three days and later transferred to a nutrient solution in a hydroponics system. After 12 days, the nutrient solution was replaced in order to set the treatments containing Pb(II) and Pb(II)/EDTA. As in the agar experiments, chelating agent-free as well as metal-free media were used as control. Concentrations of Pb(II) used were 10, 20, 40 and 80 mg l^{-1}. Plants were allowed to stay in the system for 10 days before harvesting for evaluation of Pb(II) uptake by tumbleweed plants.

Evaluation of Pb(II) effect on plant growth and Pb(II) uptake by tumbleweed plants

After harvesting, the roots and shoots of 10 plants per replicate were measured. The plants were washed with 0.01M HCl and rinsed with D.I. water. Afterwards, the plants were separated in roots, stems and leaves, oven-dried at 70ºC for 72 h and the dry biomass was recorded. The data was analyzed to determine Pb(II) effect on plant elongation and biomass accumulation. Later, samples were digested in a Perkin-Elmer microwave oven using 10 ml trace pure HNO_3. After digestion, samples were diluted in DI water (1:5) and the Pb content was determined using an inductively coupled plasma/optical emission spectrometer (ICP/OES) (Perkin-Elmer Optima 4300 DV).

Transmission Electron Microscopy sample preparation

Separated roots, stems and leaves were treated by the procedure previously published by Bozzola and Russell[10]. In summary, samples were fixed with glutaraldehyde followed

by a 1% OsO_4 solution. Subsequently, the samples were treated with uranyl acetate and dehydrated in ethanol and acetone series. Later, specimens were embedded in Poly/Bed 812 on BEEM capsules and placed on an oven at 60ºC for 48h. Ultra thin sections (60-80 nm) were cut using a Leica ultramicrotome. Sections were placed on 200 mesh copper grids and examined using a Zeiss EM10 transmission electron microscope at 60 kV.

RESULTS

Effect of Pb(II) and Pb(II)/EDTA on plant growth

The effect of Pb(II) treatments on plant growth was analyzed on plants grown in agar media only. This analysis was not performed for the hydroponics experiments due to the heterogeneity in plant size at the time of experimental setup. The results demonstrated that Pb(II) at 20 mg l^{-1} or lower did not significantly reduce the root size (between 3.0 and 3.5 cm). Moreover, none of the Pb(II) concentrations used in this study affected size of the stems (between 5.0 and 6.0 cm). The presence of EDTA did not affect root and shoot elongation. Additionally, none of the treatments affected the biomass accumulation in all the plant parts. This finding represents an advantage over some hyperaccumulators such as *Thlaspi rotundifolium* L., which is a plant species that is able to accumulate and translocate Pb but produces small biomass accumulation[9].

Effect of EDTA on Pb(II) uptake by tumbleweed plants.

Table 1 shows a summary of Pb concentrations found in tissues of tumbleweed plants cultivated in agar media (125 mg Pb l^{-1}) and hydroponics system (80 mg Pb l^{-1}) in the presence and absence of metal EDTA. This table shows that tumbleweed plants accumulated 30,000, 5,500, and 2,000 mg kg^{-1}DW in roots, stems and leaves, respectively. In addition, it was found that EDTA was efficient in enhancing Pb(II) translocation from roots to leaves. Table 1 shows that in hydroponically grown plants the concentration in leaves increased from 50 to 1421 mg kg^{-1}, in the presence of EDTA. This means that leaves concentrated 28 times more Pb when EDTA was present in the media. However, the plants cultivated in agar with EDTA accumulated 508, 812 and 800 mg Pb kg^{-1} DW in roots, stems and leaves, respectively. This means that EDTA somehow interfered with Pb uptake. It is possible that an EDTA-agar polymer was formed, which trapped Pb(II) avoiding metal absorption by tumbleweed plants. Since a Pb hyperaccumulator plant species should be able to accumulate 1,000 mg Pb kg^{-1}, these results indicate that tumbleweed could be considered a potential Pb hyperaccumulator desert plant species. In addition, the low toxicity that Pb caused on tumbleweed plants makes this a very promising plant to be used for the phytoremediation of Pb contaminated sites.

Table 1. Summary of Pb uptake and translocation in tumbleweed plants cultivated in agar and hydroponics system.

Plant tissue	Hydroponics (80 mg Pb l^{-1})		Agar (125 mg Pb l^{-1})	
	Pb[a]	Pb-EDTA[a]	Pb[a]	Pb-EDTA[a]
Root	30,000 ± 4,600	275 ± 42	25,500 ± 7,300	508 ± 80
Stem	1,500 ± 126	282 ± 65	5,500 ± 1,700	812 ± 280
Leaf	50 ± 8	1,421 ± 155	2,000 ± 440	800 ± 78

[a]Figures represent mg Pb per kg dry tissue ± C.I. 95%.

Transmission electron microscopy studies

Interesting features on cell structure were found on tumbleweed plants treated with Pb(II) in the presence and absence of EDTA. The plants treated with EDTA showed abundant vesiculation while those plants grown in an EDTA-free media did not show this vesiculation. Studies are currently being performed in order to determine if EDTA has any toxicological effect on tumbleweed plants.

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